CONTRAST AGENT

Field of invention

The present invention relates to targeted contrast agents suitable for use in diagnostic imaging techniques in which a disease state may be imaged. More specifically the invention relates to contrast agents for the imaging of diseases associated with the up-regulation of the Angiotensin II type receptor AT_1. The invention describes ligands designed to have increased potency and optimised excretion and biodistribution profiles compared to existing pharmaceutical preparations.

Diseases which can be detected by the use of a contrast agent targeting the AT_1 receptor are congestive heart failure (CHF), atherosclerosis and fibrosis in organs like heart, lungs and liver.

Background of invention

Angiotensin II (Ang II) - the octapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) - is a pleiotropic vasoactive peptide that binds to two distinct receptors: the Ang II type 1 (AT_1) and type 2 (AT_2) receptors. Activation of the renin-angiotensin aldostrone system (RAAS) results in vascular hypertrophy, vasoconstriction, salt and water retention, and hypertension. These effects are mediated predominantly by AT_1 receptors. Paradoxically, other Ang II-mediated effects, including cell death, vasodilation, and natriuresis, are mediated by AT_2 receptor activation. The understanding of Ang II signalling mechanisms remains incomplete. AT_1 receptor activation triggers a variety of intracellular systems, including tyrosine kinase-induced protein phosphorylation, production of arachidonic acid metabolites, alteration of reactive oxidant species activities, and fluxes in intracellular Ca^{2+} concentrations. AT_2 receptor activation leads to stimulation of bradykinin, nitric oxide production, and prostaglandin metabolism, which are, in large part, opposite to the effects of the AT_1 receptor. (See: Berry C, Touyz R, Dominiczak AF, Webb RC, Johns DG.: Am J Physiol Heart Circ Physiol. 2001 Dec;281(6):H2337-65. Angiotensin receptors: signalling, vascular pathophysiology, and interactions with ceramide).

Ang II is the active component of the renin-angiotensin-aldosterone system (RAAS). It plays an important physiological role in the regulation of blood pressure, plasma volume, sympathetic nervous activity, and thirst responses. Ang II also has a pathophysiological role in cardiac hypertrophy, myocardial infarction, hypertension, chronic obstructive pulmonary disease, liver fibrosis and atherosclerosis. It is produced systemically via the classical RAAS

and locally via tissue RAAS. In the classical RAAS, circulating renal-derived renin cleaves hepatic-derived angiotensinogen to form the decapeptide angiotensin I (Ang I), which is converted by angiotensin-converting enzyme (ACE) in the lungs to the active Ang II. Ang I can also be processed into the heptapeptide Ang-(1-7) by tissue endopeptidases. The RAAS system is illustrated schematically in Figure 1 hereto which is based on Figure 1 in the article by Foote et al. in Ann. Pharmacother. 27: 1495-1503 (1993).

In addition to the RAAS playing an important role in the normal cardiovascular homeostasis, over activity of the RAAS has been implicated in the development of various cardiovascular diseases, such as hypertension, congestive heart failure, coronary ischemia and renal insufficiency. After myocardial infarction (MI), RAAS becomes activated. Specifically the AT_1 receptor seems to play a prominent role in post-MI remodelling, since AT_1 receptor expression is increased after MI and in left ventricular dysfunction. Therefore drugs that interfere with RAAS, such as ACE inhibitors and AT_1 receptor antagonists, have been shown to be of great therapeutic benefit in the treatment of such cardiovascular disorders.

For heart, kidneys, lungs and liver alike, fibrosis represents a common pathway to their failure. Understanding pathophysiologic mechanisms involved in organ fibrosis is therefore of considerable interest, particularly given the potential for protective pharmacological strategies. Tissue repair involves inflammatory cells, including members of the monocyte/macrophage lineage, integral to initiating the repair process; and myofibroblasts, phenotypically transformed interstitial fibroblasts, responsible for collagen turnover and fibrous tissue formation. Each of these cellular events in the microenvironment of repair are associated with molecular events that lead to the de novo generation of angiotensin II (Ang II). In an autocrine/paracrine manner, this peptide regulates expression of TGF-beta 1 via angiotensin (AT_1) receptor-ligand binding. It is this cytokine that contributes to phenotypic conversion of fibroblasts to myofibroblasts (myoFb) and regulates myofibroblast turnover of collagen. Angiotensin-converting enzyme (ACE) inhibition or AT_1 receptor antagonism each prevent many of these molecular and cellular responses that eventuate in fibrosis and therefore have been found to be protective interventions. (See: Weber KT. Fibrosis, a common pathway to organ failure: angiotensin II and tissue repair. Semin Nephrol. 1997 Sep; 17(5):467-91 and references therein).

Ang II may regulate tissue fibrosis via the activation of mesenchymal cells. For example, Ang II stimulates the proliferation of cardiac fibroblasts *in vitro* via activation of AT_1. The presence of AT_1 receptors has also been demonstrated on cardiac fibroblasts *in vitro*. Most of the profibrotic effects of Ang II appear to be mediated via this receptor; however, increased AT_2

expression on cardiac fibroblasts has been detected in hypertrophied human heart, and the balance between the expression of these two subtypes may be critical in determining the response to Ang II. (See: Am. J. Respir. Crit. Care Med., Volume 161, Number 6, June 2000, 1999-2004Angiotensin II Is Mitogenic for Human Lung Fibroblasts via Activation of the Type 1 Receptor Richard P. Marshall, Robin J. McAnulty, and Geoffrey J. Laurent and references therein).

The Ang II receptors can be distinguished according to inhibition by specific antagonists. AT_1 receptors are selectively antagonized by biphenylimidazoles, such as Losartan, whereas tetrahydroimidazopyridines specifically inhibit AT_2 receptors. The AT_2 receptor may also be selectively activated by CGP-42112A. This is a hexapeptide analog of Ang II, which may also inhibit the AT_2 receptor, depending on concentration). Two other angiotensin receptors have been described: AT_3 and AT_4 subtypes.

In rodents, the AT_1 receptor has two functionally distinct subtypes, AT_{1A} and AT_{1B}, with >95% amino acid sequence homology.

The second major angiotensin receptor isoform is the AT_2 receptor. It has low amino acid sequence homology (~34%) with AT_{1A} or AT_{1B} receptors. Although the exact signaling pathways and the functional roles of AT_2 receptors are unclear, these receptors may antagonize, under physiological conditions, AT_1-mediated actions inhibiting cell growth and by inducing apoptosis and vasodilation. The exact role of AT_2 receptors in cardiovascular disease remains to be defined.

Other receptors for Ang II besides AT_1 and AT_2 are known and are generally referred to as $AT_{atypical}$ (see Kang et al., Am. Heart J. 127: 1388-1401 (1994)). The suppression of Ang II's effects has been used therapeutically, for example in the management of hypertension and heart failure. This has been achieved in a number of ways: by the use of renin inhibitors which block the conversion of angiotensinogen to angiotensin I (the precursor to Ang II); by the use of angiotensin converting enzyme (ACE) inhibitors that block the conversion of angiotensin I to Ang II (and also block bioconversion of bradykinin and prostaglandins); by the use of anti- Ang II -antibodies; and by the use of Ang II -receptor antagonists.

Beta blockers are most commonly used in treatment of arrhythmias. Anti-arrhythmic drugs have had limited overall success and calcium channel blockers can sometimes induce arrhythmias. No single agent shows superiority, with the possible exception of amiodarone. Short-term anti-arrhythmic benefit has been found to be offset by, depending on the specific

drug, neutral or negative effects on mortality (Sanguinetti MC and Bennett, PB: Anti-arrhythmic drug target choices and screening. Circulation 2003, 93(6): 491-9257-263). Clearly better anti-arrhythmic drugs are needed.

A publication in Lancet (Lindholm, LH et al. Effect of Losartan on sudden cardiac death in people with diabetes: data from the LIFE study. The Lancet, 2003, 362: 619-620) revealed that AT_1 receptor antagonists in addition of being generally favourable to patients with CHF, also reduce the incidence of sudden cardiac death. There exist a few studies showing that AT_1 antagonists have an anti-arrhythmia effect on arrhythmias induced by myocardial infarct or in reperfusion after ligation of LAD (Harada K et al. Angiotensin II Type 1a Receptor is involved in the occurrence of reperfusion arrhythmias. Circulation. 1998,97:315-317. Ozer MK et al. Effects of Captopril and Losartan on myocardial ischemia-reperfusion induced arrhythmias and necrosis in rats. Pharmacological research, 2002, 45 (4), 257-263 Lynch JJ et al. EXP3174, the AII antagonist human metabolite of Losartan, but not Losartan nor the Angiotensin-converting enzyme inhibitor captopril, prevents the development of lethal ischemic arrhythmias in a canine model of recent myocardial infarction. JACC, 1999, 34 876-884).

It has now been found that it is possible to image Ang II receptor sites in vivo using targeted contrast agents in which the targeting binding ligand has affinity for Ang II-receptor sites. The Ang II receptors are generally accessible to such contrast agents when they are administered into the blood stream. Accordingly, using such targeted contrast agents it is possible to detect diseases and disorders such as heart failure, atherosclerosis and restricted blood flow, as well as other vascular diseases and disorders, and also to monitor the progression of treatment for such diseases and disorders.

Description of Related Art

WO 98/18496 (Nycomed Imaging AS) discloses contrast agents comprising Ang II-receptor antagonists labelled for in vivo imaging.

US patent no. 5,138,069 discloses substituted imidazoles for use as Ang II receptor blockers. Further, US patent no. 5,264,581 (Cariani) discloses radioiodinated imidazole Ang II antagonists.

Summary of the invention

When using imidazoles, e.g. Losartan, as the binding ligand in a conjugate with a relatively large reporter, such as a bulky chelate, the affinity of the ligand for the selected binding site may be negatively affected.

A problem with agents like Losartan, both the drug and the chelate conjugate, is that they excrete mainly (more than 80 %) through the liver and have an affinity (Ki) which is less avid than the natural hormone Ang II. This constitutes two problems in the use of such compositions as targeting contrast agents: Firstly a small amount of the administered composition is allowed to bind to the Ang II receptor site before it is absorbed in the liver and secondly the liver up-take results in increased background activity e.g. the background from the liver may interfere with imaging of diseased areas of the heart.

It has now been found that the introduction of an amino acid-comprising biomodifier/linker between the targeting ligand and the chelate or reporter moiety can reduce the liver up-take and can also increase the binding affinity to Ang II receptor sites. The biomodifier/linker may be linear or branched. Hence, compositions of matter of the present invention are useful diagnostic contrast agents for *in vivo* imaging of the mammalian body. Furthermore, the patients undergoing the imaging procedure may also be on 'sartan' drug therapy. 'Sartans' e.g. Losartan are Ang II receptor antagonists used in treatment of hypertension. A contrast agent targeting Ang II receptors will experience a competition with the treatment drug for binding to the same receptor site. It is therefore considered desirable to develop contrast agents possessing higher affinity for the AT_1 receptor than the prescribed drug to avoid deleterious competition effects of the cold drug i.e. the prescribed non-detectable 'sartan' drug.

The *in vivo* detectable moiety (reporter) can be any moiety capable of detection either directly or indirectly in an *in vivo* diagnostic imaging procedure e.g. by MRI, optical imaging, scintigraphy, SPECT, PET , X-ray, ultrasound, electrical impedance or magnetometric procedures.

The compositions of matter of the present invention are useful for the *in vivo* diagnostic imaging of a range of disease states (congestive heart failure (CHF), artherosclerosis, fibrosis in organs like heart, lungs and liver) where the up regulation of Ang II receptor sites is known to be involved.

Detailed description of the invention

In a first aspect the present invention provides a composition of matter of formula I

V - L - R (I)

where V is an organic group having binding affinity for an angiotensin II receptor site, L is an amino acid-comprising biomodifier or linker moiety, and R is a moiety detectable in an *in vivo* imaging procedure of a human or animal body.

The ligand V may be any organic compound having affinity for Ang II receptors. Compounds having a marked affinity for particular types of Ang II receptors, such as AT_1 or AT_2, are generally preferred. Imidazole Ang II antagonist ligands are preferred and most preferred are ligands such as Losartan, Valsartan, Candesartan and Eprosartan.

For contrast agents useful in diagnosis and particularly in *in* vivo diagnosis the moiety R must be able to carry the imageable moiety or moieties denoted M. By carrying is meant any form of association between the moiety R and M such as a chemical bond, e.g. covalent bond or electrovalent or ionic bonds or by absorption or any other type of association. R can be any imageable moiety. Where M is a metal entity then Y_1 represents a chelating agent. The nature of R and/or Y_1M will depend of the imaging modality utilised in the diagnosis. R and/or Y_1M must be capable of detection either directly or indirectly in an *in vivo* diagnostic imaging procedure, and comprise e.g. moieties which emit or may be caused to emit detectable radiation (eg. by radioactive decay, fluorescence excitation, spin resonance excitation, etc.), moieties which affect local electromagnetic fields (eg. paramagnetic, superparamagnetic, ferrimagnetic or ferromagnetic species), moieties which absorb or scatter radiation energy (eg. chromophores, particles (including gas or liquid containing vesicles), heavy elements and compounds thereof, etc.), and moieties which generate a detectable substance (eg. gas microbubble generators).

In a preferred embodiment one moiety R is covalently bound directly to L forming an N-alkyl glycine unit.

Chelating agents of formula (II) and (e) hereinafter are also particularly preferred.

A wide range of suitable imageable moieties are known from e.g. WO 98/18496, the content of which is incorporated by reference.

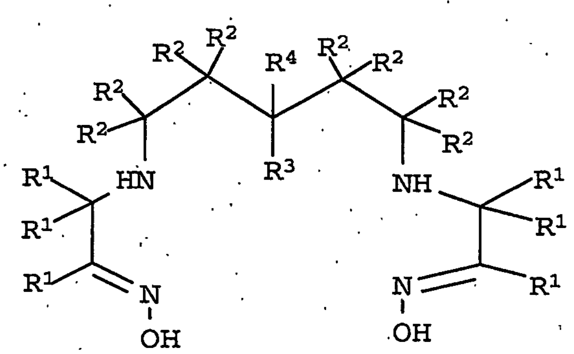
Imaging modalities and imageable moieties R and M are described in more detail hereinafter:

In a first embodiment, the compound of formula (I) comprises a moiety Y_1 carrying one or more imageable moieties M useful in the Radio and SPECT imaging modality. Preferably M is a gamma emitter with low or no alpha- and beta-emission and with a half-life of more than one hour. Preferred groups M are the radionuclides ^{67}Ga, ^{111}In, ^{123}I, ^{125}I, ^{131}I, ^{81m}Kr, ^{99}Mo, ^{99m}Tc, ^{201}Tl and ^{133}Xe. Most preferred is ^{99m}Tc.

M can further be represented by the following isotopes or isotope pairs for use both in imaging and therapy without having to change the radiolabeling methodology or chelator: $^{47}Sc_{21}$; $^{141}Ce_{58}$; $^{188}Re_{75}$; $^{177}Lu_{71}$; $^{199}Au_{79}$; $^{47}Sc_{21}$; $^{131}I_{53}$; $^{67}Cu_{29}$; $^{131}I_{53}$ and $^{123}I_{53}$; $^{188}Re_{75}$ and $^{99m}Tc_{43}$; $^{90}Y_{39}$ and $^{87}Y_{39}$; $^{47}Sc_{21}$ and $^{44}Sc_{21}$; $^{90}Y_{39}$ and $^{123}I_{53}$; $^{146}Sm_{62}$ and $^{153}Sm_{62}$; and $^{90}Y_{39}$ and $^{111}In_{49}$.

When M denotes a metallic radionuclide then Y_1 denotes a chelating agent suitable for forming a stable chelate with M. Such chelating agents are well known from the state of art and typical examples of such chelating agents are described in Table I of WO 01/77145.

Particularly preferred are chelating agents of formula (II):

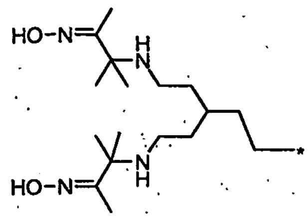


(II)

wherein:

each R^1, R^2, R^3 and R^4 is independently H or C_{1-10} alkyl, C_{3-10} alkylaryl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} alkylamine, C_{1-10} fluoroalkyl, or 2 or more R groups, together with the atoms to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring.

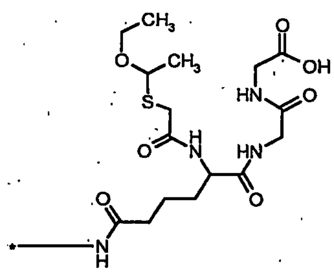
More particularly preferred are chelating agents of formula (II) where R^1, R^2 and R^3 are hydrogen or methyl groups and R^4 is an alkylamine group, most specifically a compound of formula (e), herein denoted cPN216. For the following structures the asterisks denote possible linking sites. For formula (e) the asterisk denotes an amine group.



Formula (e)

Most preferred for Y_1 is when the chelate is cPN216 and when the imaging moiety M is ^{99m}Tc.

Other preferred chelating agent can be represented by formulas a, b, c and d.



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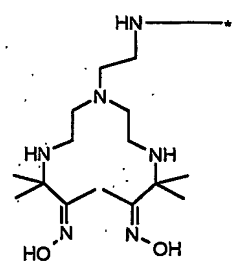

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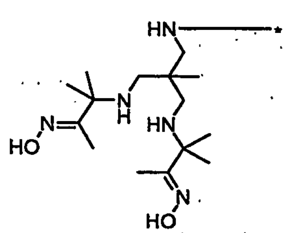

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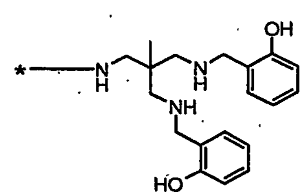
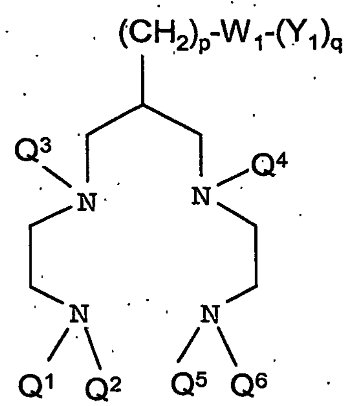


d

Synthesis of chelating agents of formula (II) and (e) are described in WO 03/006070.

Other relevant chelating agents are of formula (III)



(III)

wherein Q_1-Q_6 are independently Q groups, where Q is H, alkyl, aryl or an amine protecting group,
W_1 is –NR- , –CO_2- , -CO- , -NR(C=S)- , -NR(C=O)-, –CONR-
or a Q group;
each Y is independently a *D*- or *L*- amino acid, -CH_2- , –CH_2OCH_2- or –OCH_2CH_2O- or a W_1 group;
p is an integer of value 1 to 8;
q is an integer of value 0 to 30;
R is H, C_{1-4} alkyl, C_{2-4} alkoxyalkyl, C_{1-4} hydroxyalkyl, or C_{1-4} fluoroalkyl;

Synthesis of tetraamin chelting agents of formula (III) can be found in GB patent application number GB 0416062.8.

Non-metal radionuclides such as ^{123}I, ^{125}I and ^{131}I may be covalently linked to the moiety L by a substitution or addition reaction well known from the state of art.

In a second embodiment, the compound of formula (I) comprises a moiety R useful in the PET imaging modality. R then denotes a radioemitter with positron-emitting properties. Preferred groups R are the radionuclides ^{11}C, ^{18}F, ^{68}Ga, ^{13}N, ^{15}O and ^{82}Rb. ^{18}F is specifically preferred. The metallic radioemitters ^{82}Rb and ^{68}Ga chelated with a chelating agent Y_1 are also preferred.

Thiol coupling chemistry, ^{18}F-synthons and labelled peptides prepared using the thiol coupling chemistry are described in WO 03/080544, the content of which is incorporated herein by reference.

Description of peptides labelled by use of thiol coupling chemistry can be found in GB patent application no. 0317815.9, the content of which is incorporated herein by reference.

When M denotes a metallic radionuclide then Y_1 denotes a chelating agent suitable for forming a stable chelate with M. Such chelating agents are well known from the state of art and typical examples of such chelating agents are described in Table I of WO 01/77145 and to the previous part on Radio and SPECT imaging.

In another preferred embodiment Y_1 is the DOTA chelating agent and M is ^{68}Ga which can be readily introduced in to the chelate using microwave chemistry.

Non-metal radionuclides such as ^{18}F may be covalently linked to the moiety L by a substitution or addition reaction well known from the state of art and also described eg. in WO03/080544 which is hereby incorporated by reference.

In a third embodiment, R of the compound of formula (I) comprises a moiety Y_1carrying one or more imageable moieties M useful in the MR imaging modality. M here denotes a paramagnetic metal such those mentioned in US patent 4 647 447, Gd^{3+} , Dy^{3+}, Fe^{3+} and Mn^{2+} are particularly preferred and Y_1 denotes a chelating agent, in particular a chelating agent such as acyclic or cyclic polyaminocarboxylates (e.g. DTPA, DTPA-BMA, DOTA and DO3A) as described e.g. in US patent 4 647 447 and WO 86/02841. M may also denote metal oxides such as superparamagnetic, ferrimagnetic or ferromagnetic species which are absorbed by R, e.g. such that R functions as a coating to the metal oxide. Metal oxides for use as MR contrast agents are described e.g. in US patent 6 230 777 which is hereby incorporated by reference.

In a fourth embodiment R of the compound of formula (I) comprises a moiety Y_1 carrying one or more imageable moieties M useful in the X-ray imaging modality. M here denotes a heavy metal such as W, Au and Bi preferably in the form of oxides which may be absorbed to R. R can also be represented by iodinated aryl derivatives particularly well known as X-ray contrast agents, e.g. Iopamiron™ and Omnipaque™. These agents can e.g. be linked via a amide or amine function to V of formula (I).

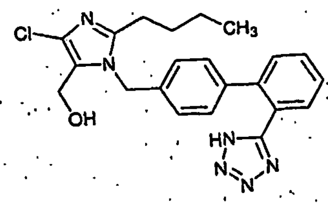
In a further embodiment the compound of formula (I) comprises R in the form of gas filled microvesicles. Such ultrasound imaging agents can be utilised in the imaging of receptors e.g. when they are functionalised for binding to a peptide as described in the state of art e.g. in WO98/18500.

In a sixth embodiment of the present invention the moiety R of formula (I) may be any moiety capable of detection either directly or indirectly in an optical imaging procedure. The detectable moiety can be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter. More preferably R is represented by a dye such as a chromophore or a fluorescent compound. The moiety R can be any dye that interacts with light in the electromagnetic spectrum with wavelengths from the ultraviolet light to the near-infrared. In a preferred version R has fluorescent properties.

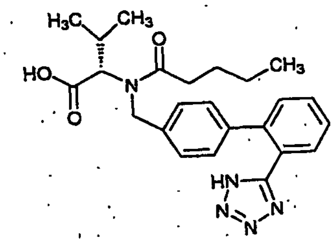
Preferred organic dye moieties include groups having an extensive delocalized electron system, eg. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyrilium dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, napthoquinones, indathrenes, phthaloylacridones, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, bis(dithiolene) complexes, bis(benzene-dithiolate) complexes, iodoaniline dyes, bis(S,O-dithiolene) complexes. Fluorescent proteins, such as green fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium, samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots).

Further descriptions of moieties suitable in optical imaging procedures are found in Norwegian patent application no. 200303115 the content of which is hereby incorporated by reference.

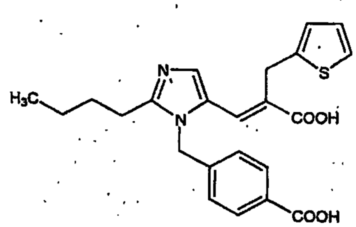
The invention may be exemplified by Losartan derivatives and is based on attachment of biomodifier/linker and reporter moieties to the imidazol 5-position. The principle also applies to other compounds having structural similarities, e.g. Valsartan, Candesartan and Eprosartan, possessing suitable anchoring sites in the part of the molecule corresponding to the Losartan imidazole ring.



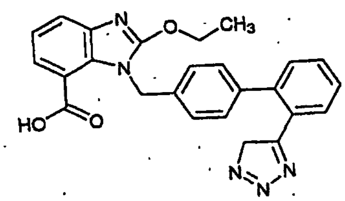
Losartan



Valsartan



Eprosartan



Candesartan

One role of the biomodifier/linker moiety L may be to distance a relatively bulky reporter, such as a metal complex, from the active site of the binding ligand V. The biomodifier/linker moiety can be selected to increase the binding affinity of the composition for the receptor. The biomodifier/linker moiety comprises 1-40 amino acids, and preferred 1-20 amino acids, and more preferred 1-10 amino acids and most preferred 1-5 amino acids. Further the biomodifier/linker moiety may comprise one or more dicarboxylic acid units (e.g. diglycoloyl units, glycolyl units, succinyl, glutaryl units), ethyleneglycol units, diamines, PEG or PEG like units or combinations of the above.

The nature of the linker group can also be used to modify the biodistribution of the resulting metal complex of the conjugate e.g. the introduction of amino acids with different properties can decrease the liver up-take.

Some of the compounds of the present invention have high affinity for the AT_1 receptor. "High affinity" refers to compounds having a Ki $\leq$ 5nM and preferably < 0.1 nM and most preferred Ki's in the pM or sub pM range calculated from competitive binding assays for AT_1

and where the Ki value was determined by competition with the known high affinity vector ^{125}I-Sar$_1$Ile$_8$ –angiotensin II. The Ki for Ang II in this assay system is around 5 nM.

Ang II-receptor antagonists derived from the so called 'sartan' class of drugs such as Valsartan, Candesartan and Eprosartan and preferably Losartan, labelled with an imaging moiety are useful diagnostic imaging agents for in vivo imaging of a human or animal body.

One preferred embodiment of the invention is the ^{99m}Tc labelled contrast agents ^{99m}Tc (Losartan-Leu-diglycolyl-cPn216), ^{99m}Tc (Losartan-Leu-Gly-diglycolyl-cPn216), ^{99m}Tc (Losartan-Leu-β-Ala-diglycolyl-cPn216) and ^{99m}Tc (Losartan-Leu-Lys(Propionyl-PEG(12)-Ac)-Diglycoloyl-cPn216).

The contrast agent of formula (I) are preferably administered as a pharmaceutical formulation comprising the compound of formula (I) in a form suitable for administration to a mammal, such as a human. The administration is suitable carried out by injection or infusion of the formulation such as an aqueous solution. The formulation may contain one or more pharmaceutical acceptable additives and/or excipients e.g. buffers; solubilisers such as cyclodextrins; or surfactants such as Pluronic, Tween or phospholipids. Further, stabilisers or antioxidants such as ascorbic acid, gentisic acid or para-aminobenzoic acid and also bulking agents for lyophilisation such as sodium chloride or mannitol may be added.

The present invention also provides a pharmaceutical composition comprising an effective amount (e.g. an amount effective for enhancing image contrast in an *in vivo* imaging procedure) of a composition of general formula I or a salt thereof, together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

Viewed from a further aspect the invention provides the use of a composition of formula I for the manufacture of a contrast medium for use in a method of diagnosis involving administration of said contrast medium to a human or animal body and generation of an image of at least part of said body.

Viewed from a still further aspect the invention provides a method of generating enhanced images of a human or animal body previously administered with a contrast agent composition comprising a composition of matter as defined by formula I, which method comprises generating an image of at least part of said body.

The invention further provides a method for the monitoring of the effect of treatment of heart failure and other diseases associated with up-regulation of the AT_1 receptor.

In still another aspect the invention provides a kit for the preparation of a radiopharmaceutical composition of formula (I) comprising a ligand-chelate conjugate and a reducing agent. Preferably the reducing agent is a stannous salt. The kit may further comprise one or more stabilisers, antioxidants, bulking agents for lyophilisation and solubilisers.

The three letter abbreviations used herein for the amino acids have the following meaning:

Ala - Alanine
Asp - Aspartic acid
Arg - Arginine
Glu - Glutamic acid
Gly - Glycine
Lys - Lysine
Leu - Leucine
Sar - Sarcosine
Val - Valine
Tyr - Tyrosine
Ile - Isoleucine
His - Histidine
Pro - Proline
Phe - Phenylalanine
Nal - 2- Amino-3-naphtyl propionic acid
Cha - 2- Amino-3-cyclohexyl propionic acid

The meaning of other abbreviations used herein is as follow:

DOTA - 1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid
PEG - polyethyleneglycol
DIEA - diisopropylethylamine
DPPA - diphenylphosphoryl azide
DBU - 1,8 –diaza-bicyclo (5,4,0) undec-7- ene
DMF - dimethyl formamide
MDP - methylene diphosphonate
TFA - trifluoroacetic acid
THF - tetrahydro furan

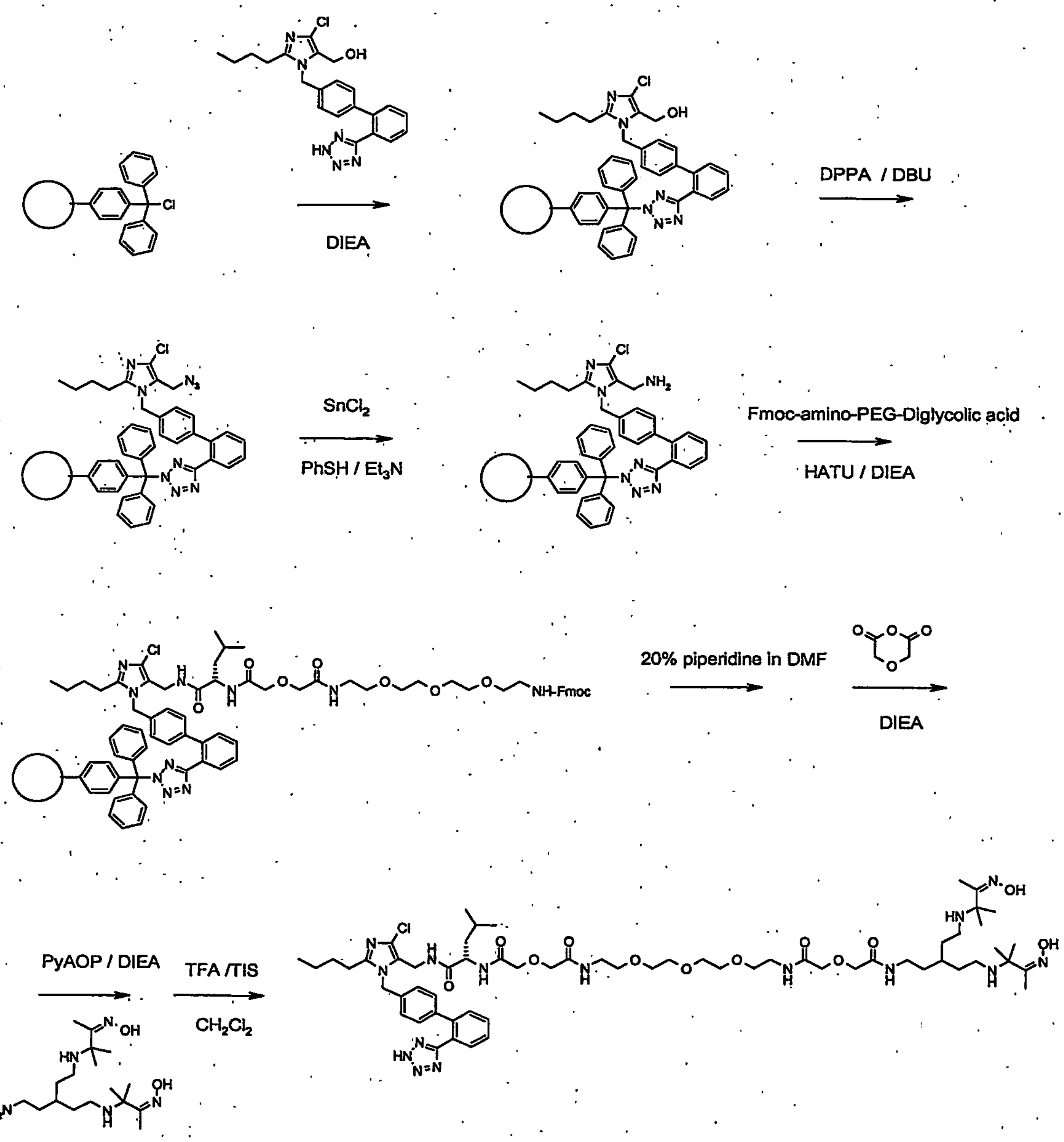
HATU - N-[(dimethylamino)- 1H- 1,2,3 – triazolo [4,5-b]pyridino-1-ylmethylene]-N-methylmethanaminium hexafluorophosphonate N-oxide

PyAOP -7-Azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium-hexafluorophosphate

Fmoc - 9-fluorenylmethoxycarbonyl

General procedures

Scheme 1 shows the solid phase synthesis of Losartan linker chelating agent conjugate. The circle donates a solid support particle.



Scheme 1

K_i determination

The affinity of the compounds was determined as the dissociation constant (K_d) and measured by displacement of a radiolabelled ligand of known affinity.

The affinity of the compound for the AT_1 receptor has been determined in a competition assay using membranes from CHO cells expressing the receptor. Binding of ^{125}I-Sar$_1$-Ile$_8$-Angiotensin II, a ligand known to bind very efficiently to AT_1. receptors, was competed with various concentrations of the test substance. The K_i is the concentration of the competing ligand in the competition assay which would occupy 50% of the receptors if no radoligand was present. K_i is calculated using the Cheng-Prussoff equation:

$$K_i = IC_{50} / (1 + (L)/K_d)$$

Where (L) is the concentration of the radiolabelled ligand used and the Kd is the dissociation constant of the radiolabelled ligand for the receptor. IC_{50} is the concentration of competing ligand which displaces 50% of the specific binding of the radioligand. The IC_{50} value for a compound may vary between experiments depending on the radioligand concentration, whereas the K_i is an absolute value.

^{99m}Tc-Labelling protocol

A preparation is made by dissolving 0,1 mg freeze-dried cPn216 derived compound in 0,2 ml (distilled and oxygen-free) water. This solution is transferred into a 10 ml nitrogen filled vial. 0,5 ml carbonate buffer, 0,5 ml $Na^{99m}TcO_4$ solution and 0,1 ml Sn-MDP solution are added. The preparation is left at room temperature for 20 minutes.

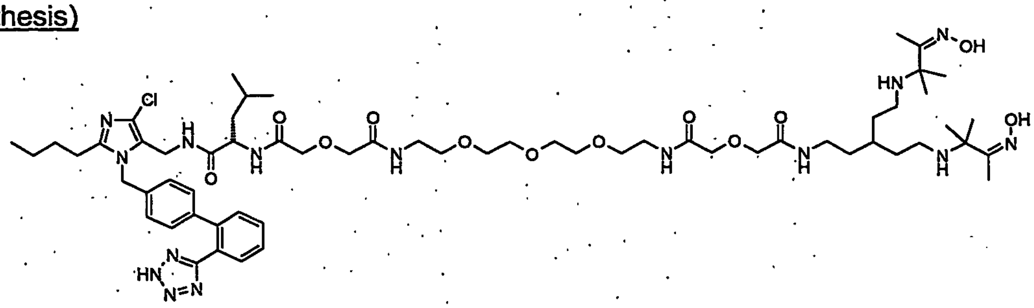
Carbonate buffer: The carbonate buffer has a pH of 9,2 and contains 8,4 mg $NaHCO_3$ and 10,6 mg Na_2CO_3 per ml water. It is purged with nitrogen gas for at least 15 minutes before use.

$Na^{99m}TcO_4$ solution: Technetium generator (e.g. Ifetec generator) eluate, diluted to a radioactive concentration of 2 GBq/ml, oxygen free.

Sn-MDP solution: This solution contains 0,131 mg $SnCl_2{*}2H_2O$ and 0,925 mg MDP (methylene diphosphonate) per ml water. The solution is made freshly before use under continuous nitrogen gas purging.

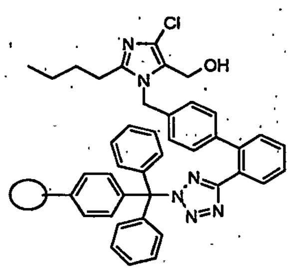
Examples

Example 1 Losartan-Leu derivatised with cPn216 via a short PEG-linker (solid phase synthesis)



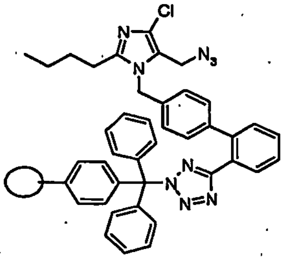
All reactions were carried out in a manual nitrogen bubbler apparatus.

a) Attachment of losartan to trityl derivatised solid support



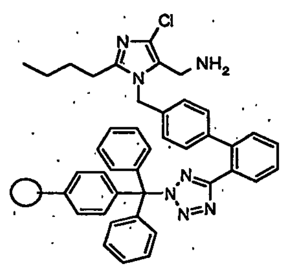
Losartan (MSD, 0.236 g, 0.558 mmol) and triethylamine (Fluka, 0.233 ml, 1.67 mmol) were added to a suspension of trityl chloride resin (Novabiochem, susbstitution 1.24 mmol/g, 0.300 g) in DMF (5 ml). After 4 days the resin was drained and washed. An aliquot of the resin was cleaved (dichloromethane/ TFA/ triisopropylsilane, 92.5: 5.0: 2.5, 15 min). HPLC analysis (column Phenomenex Luna C18(2) 3 μm 4.6 x 50 mm, solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 10-40% B over 10 min; flow 2.0 ml/min, UV detection at 214 and 254 nm) gave a peak with t_R 6.7 minutes corresponding to losartan. The resin was treated with dichloromethane/ methanol/ diisopropylethylamine solution (17 : 2 : 1, 20 ml, 1 h), washed with dichloromethane and dried.

b) Replacement of the hydroxyl group by azide



Diphenylphosphoryl azide (Aldrich, 0.481 ml, 2.23 mmol) and DBU (0.611 ml, 4.09 mmol) were added to a suspension of resin bound losartan from a) (0.372 mmol) in THF (10 ml). The reaction was left over night. An aliquot of the resin was cleaved as described under a). Analysis by LC-MS (column Phenomenex Luna C18(2) 3 μm 50 x 4.60 mm, solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 20-80% B over 10 min; flow 1 ml/min, UV detection at 214 nm, ESI-MS) gave a peak, t_R 7.3 minutes, with m/z 448.1 (MH^+) corresponding to the structure.

c) Reduction of the azide group to amine



To a suspension of the resin from b) in THF (4 ml) was added addition of tin(II)chloride (Acros, 0.141 g, 0.744 mmol), thiophenol (Fluka, 0.304 ml, 2.976 mmol) and triethylamine (Fluka, 0.311 ml, 2.23 mmol). After 1.5 hour an aliquot of the resin was cleaved as described under a). LC-MS analysis (column Phenomenex Luna C18(2) 3 μm 50 x 4.60 mm, solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 20-80% B over 10 min; flow 1 ml/min, UV detection at 214 nm, ESI-MS) gave a peak at 1.9 minutes with m/z 422.2 (MH^+) as expected for amine.

d) Losartan-Leu-Diglycoloyl-PEG(4)-Diglycoloyl-cPn216

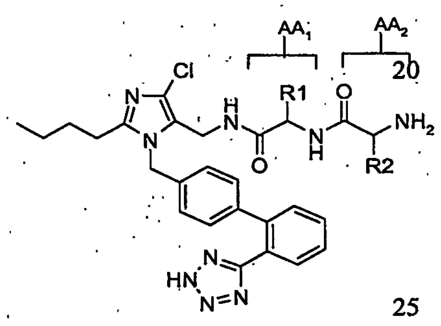
Fmoc-Leu-OH (Novabiochem, 0.030 g, 0.084 mmol) and Fmoc-amino PEG diglycolic acid (Polypure, 0.045 mg, 0.084 mmol) were successively coupled to an aliquot of the resin bound amino-losartan from c) (0.042 mmol) in DMF using standard coupling reagents (HATU and DIEA) and standard Fmoc-cleavage protocol (20% piperidine in DMF). Completion of couplings were checked by standard Kaiser test. The second diglycoloyl unit was introduced using diglycolic anhydride (Aldrich, 0.010 g, 0.084 mmol) and DIEA (0.014 ml, 0.084 mmol). To the resin (containing a terminal carboxyl function) was added the chelate cPn216 (0.029 g, 0.084 mmol), PyAOP (Applied Biosystems, 0.022 g, 0.042 mmol) and DIEA (0.014 ml, 0.084 mmol). After two hours the compound was cleaved off the resin (dichloromethane/

TFA/triisopropylsilane, 92.5 : 5.0 : 2.5 solution for 30 minutes). The solution was filtered, concentrated and purified by preparative HPLC (column Phenomenex Luna C18(2) 5 μm 10.0 x 250 mm, solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 25-30% B over 60 min; flow 5.0 ml/min, UV detection at 214 nm) to give 3 mg of product after lyophilisation. LC-MS analysis (column Phenomenex Luna C18(2) 3 μm 50 x 4.60 mm, solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 10-80% B over 10 min; flow 0.3 ml/min, UV detection at 214 and 254 nm, ESI-MS) t_R 5.9 minutes, m/z 1266.5 (MH^+)) confirmed the structure.

The compound was tested *in vitro* for binding to angiotensin-II receptor AT1 (Ki 0.5 nM).

Examples 2-18 Amino acid substituted losartan derivatives

Amino derivatives of the general formula (IV) listed in Table 1 were synthesised on solid support as described in Example 1. The products were purified by reverse phase chromatography (Phenomenex Luna C18(2) columns) using suitable gradients of acetonitrile/water containing 0.1% TFA and were analysed by LC-MS using electrospray ionisation.



(IV)

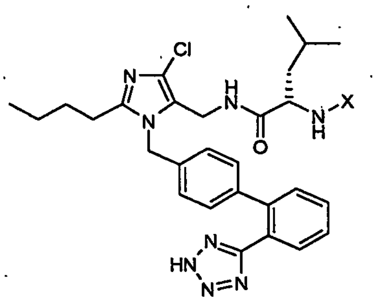
Table 1

AA1	AA2	MW (exact)	MH^+ observed
Lys		549.27	550.2
Glu		550.22	551.1
Leu		534.26	535.1
D-Lys		549.27	550.2
D-Glu		550.22	551.2
D-Leu		534.26	535.3
Leu	Lys	662.36	663.3
Leu	Glu	663.30	664.2
Leu	Leu	647.35	648.2

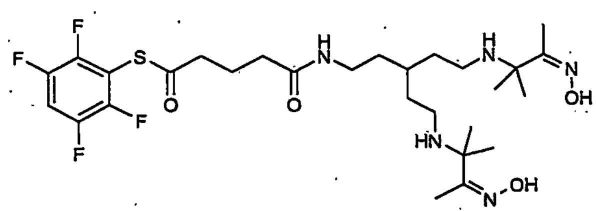
D-Leu	D-Lys	662.36	663.7
D-Leu	D-Glu	663.30	664.6
D-Leu	D-Leu	647.35	648.7
Leu	D-Leu	647.35	648.7
D-Leu	Leu	647.35	648.7
Phe		568.25	569.7
Nal		618.26	619.6
Cha		574.29	575.7

Examples 19-46 Losartan-Leu derivatives comprising a reporter moiety

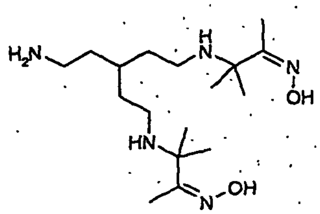
Compounds of the general formula (V) are listed in Table 2 and were synthesised on solid support as described in Example 1. Biotin and fluorescein-NHS ester were purchased from Fluka and Pierce, respectively. In the cases where Tc chelate cPn216 is attached via a glutaryl linker three different synthetic methods were used. In most cases an active ester of formula (VI) was used. As an alternative the corresponding free acid was coupled using reagents like PyAOP or HATU, but in that case the coupling had to repeated several times. Also, the glutaryl-cPn216 moiety was attached in a two step reaction, as described for the corresponding diglycoloyl derivative in Example 1, by first reacting the resin with glutaric anhydride and in a subsequent step activate the resin bound carboxylic acid and couple cPn216 as the free amine of formula (VII). For synthesis of compounds containing the tetraamine chelate the fully Boc-protected building block (VIII) (GB 0416062.8) was used. The products were purified by reverse phase chromatography (Phenomenex Luna C18(2) columns) using suitable gradients of acetonitrile/water containing 0.1% TFA or formic acid and were analysed by LC-MS using electrospray ionisation.



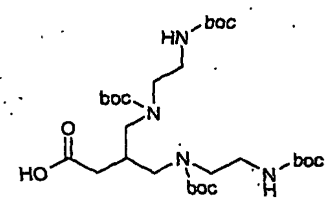
(V)



(VI)

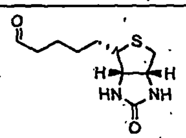
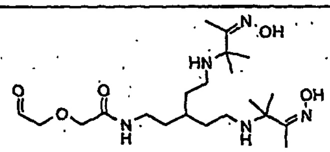
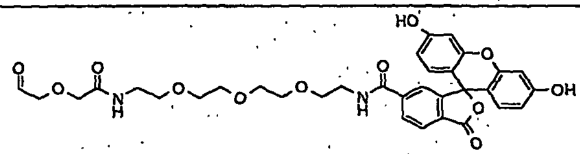
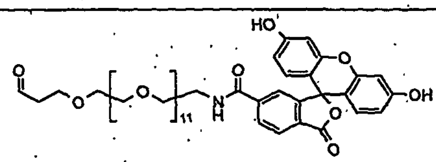
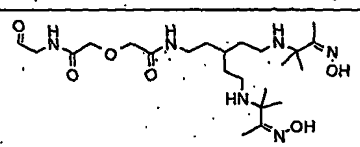
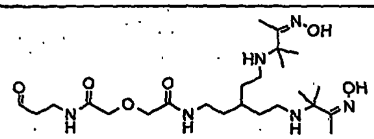
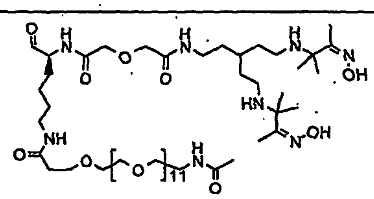


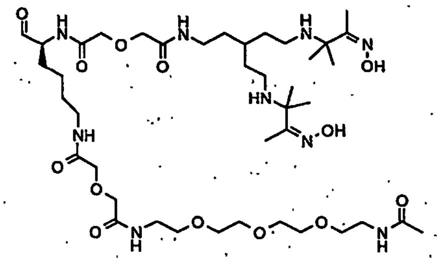
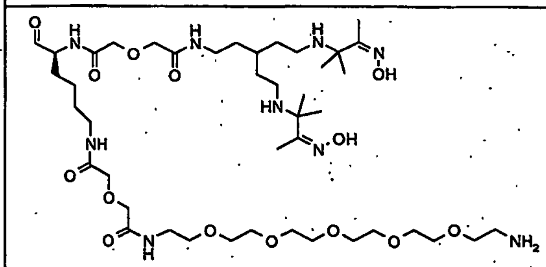
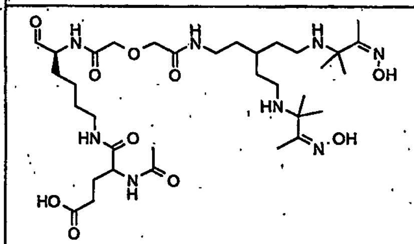
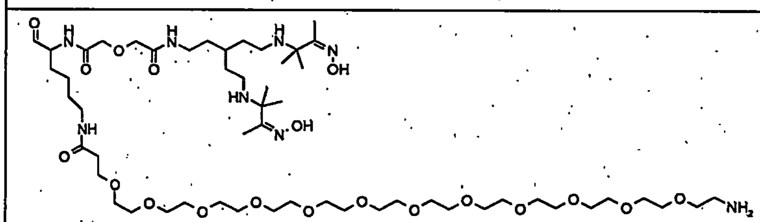
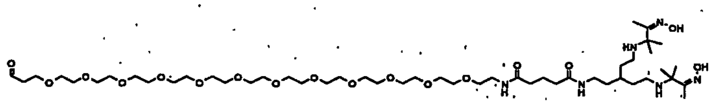
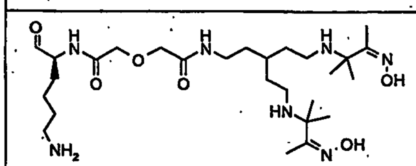
(VII)

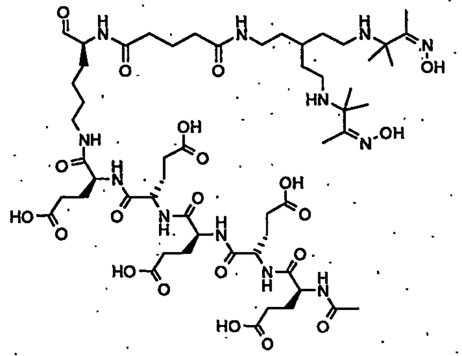
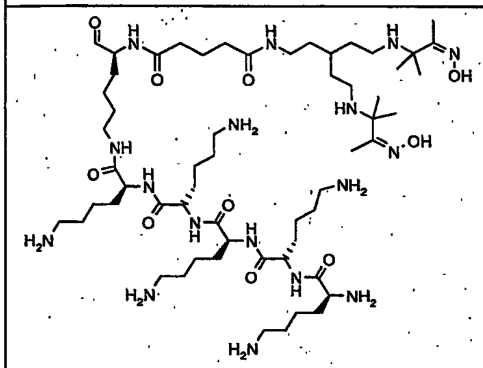
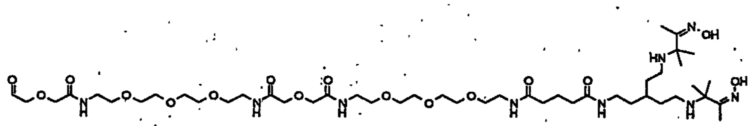
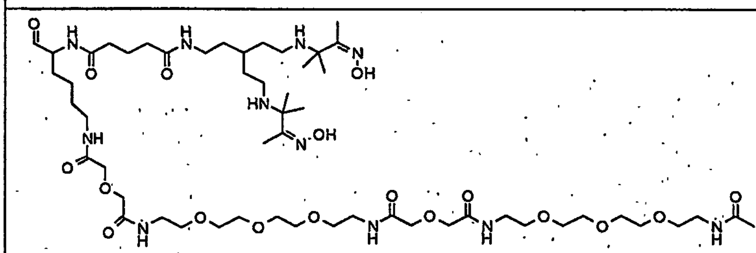
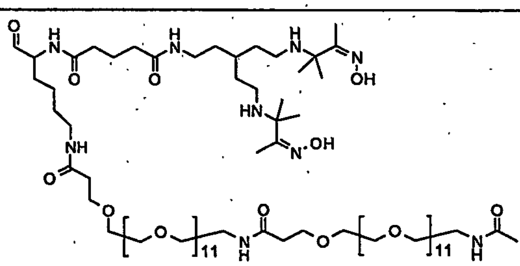
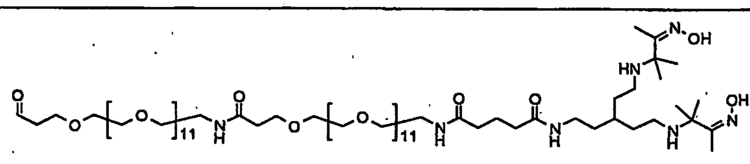


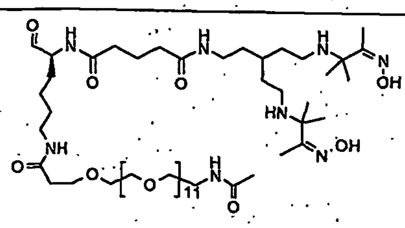
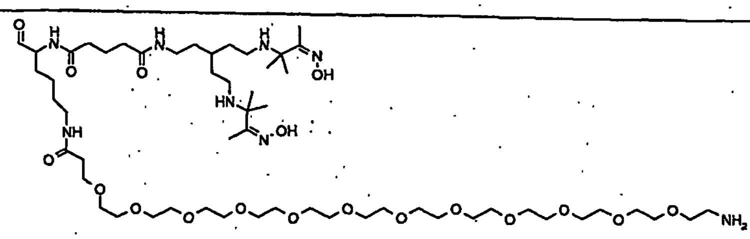
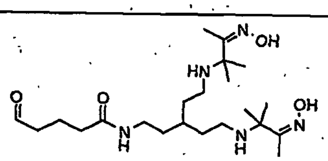
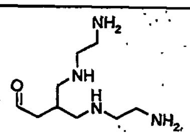
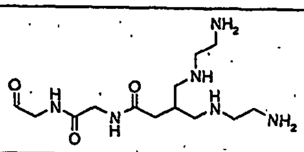
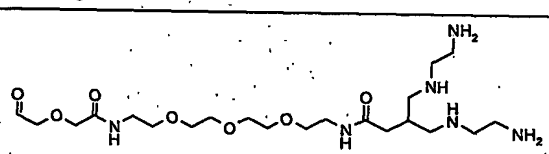
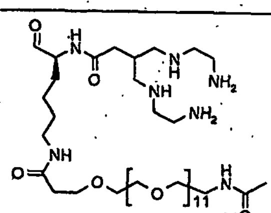
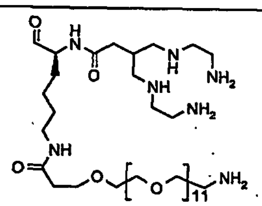
(VIII)

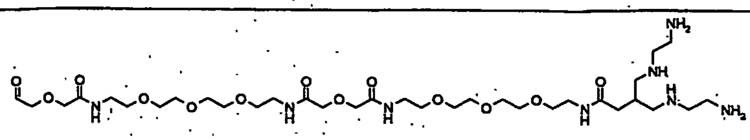
Table 2

X	Abbr	MW (exact)	MH+ obs.
	Losartan-Leu-Biotin	760.34	761.6
	Losartan-Leu-Diglycoloyl-cPn216	975.56	975.9
	Losartan-Leu-Diglycoloyl-PEG(4)-Fluorescein	1182.46	1183.4
	Losartan-Leu-Propionyl-PEG(12)-Fluorescein	1491.66	1492.6
	Losartan-Leu-Gly-Diglycoloyl-cPn216	1032.58	1033.7
	Losartan-Leu-β-Ala-Diglycoloyl-cPn216	1046.59	1047.5
	Losartan-Leu-Lys(Propionyl-PEG(12)-Ac)-Diglycoloyl-cPn216	1745.01	1745.6

	Losartan-Leu-Lys(Diglycoloyl-PEG(4)-Ac)-Diglycoloyl-cPn216	1435.81	719.1 (MH_2^{2+})
	Losartan-Lys(Diglycoloyl-PEG(6)-NH2)-Diglycoloyl-cPn216	1481.85	742.1 (MH_2^{2+})
	Losartan-Leu-Lys(Glu-Ac)-Diglycoloyl-cPn216	1274.71	1275.5
	Losartan-Leu-Lys(Propionyl-PEG(12)-NH2)-Diglycoloyl-cPn216	1703.00	852.6 (MH_2^{2+})
	Losartan-Leu-Propionyl-PEG(12)-Glutaryl-cPn216	1572.93	787.6 (MH_2^{2+})
	Losartan-Leu-Lys-Diglycoloyl-cPn216	1103.65	552.4 (MH_2^{2+})

	Losartan-Leu-Lys (Glu_5-Ac)-Glutaryl-cPn216	1788.90	895.9 (MH_2^{2+})
	Losartan-Leu-Lys (Lys_5)-Glutaryl-cPn216	1742.15	872.5 (MH_2^{2+})
	Losartan-Leu-[Diglycoloyl-PEG(4)]2-Glutaryl-cPn216	1553.87	778.1 (MH_2^{2+})
	Losartan-Leu-Lys([Diglycoloyl-PEG(4)]2-Ac)-Glutaryl-cPn216	1723.98	863.2 (MH_2^{2+})
	Losartan-Leu-Lys([Propionyl-PEG(12)]2-Ac)-Glutaryl-cPn216	2342.39	1172.9 (MH_2^{2+})
	Losartan-Leu-[Propionyl-PEG(12)]2-Glutaryl-cPn216	2172.28	1087.2 (MH_2^{2+})

	Los-Leu-Lys(Propionyl-PEG12-Ac)-Glutaryl-cPn216	1743.04	1743.7
	Losartan-Leu-Lys(Propionyl-PEG(12)-NH2)-Glutaryl-cPn216	1701.02	852.0 (MH_2^{2+})
	Losartan-Leu-Glutaryl-cPn216	973.58	974.5
	Los-Leu-Tetraamine	734.43	735.5
	Los-Leu-Gly-Tetraamine	848.47	849.3
	Losartan-Leu-Diglycoloyl-PEG(4)-Tetraamine	1024.57	1025.5
	Los-Leu-Lys(Propionyl-PEG12-Ac)-Tetraamine	1503.88	1504.7
	Losartan-Leu-Lys(Propionyl-PEG(12)-NH2)-Tetraamine	1461.87	1462.7

	Losartan-Leu-Lys-[Diglycoloyl-PEG(4)]2-Tetraamine	1314.72	658.7 (MH_2^{2+})